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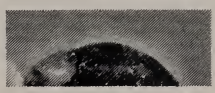
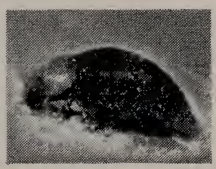
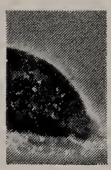

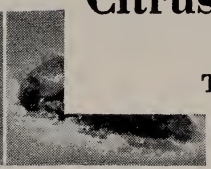

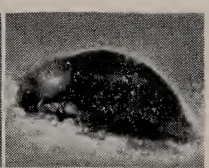

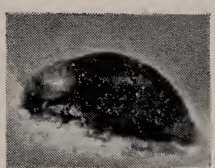

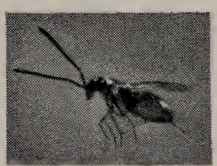
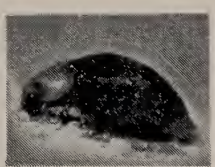

T. W. FISHER

Mass Culture of *Cryptolaemus* and *Leptomastix*—Natural Enemies of Citrus Mealybug

CALIFORNIA AGRICULTURAL EXPERIMENT STATION

BULLETIN 797

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JULY, 1963

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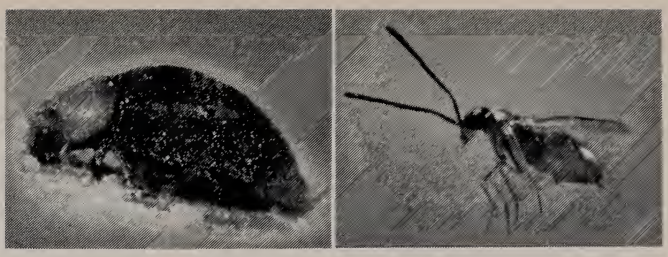


Mass Culture of *Cryptolaemus* and *Leptomastix* — Natural Enemies of Citrus Mealybug [1]

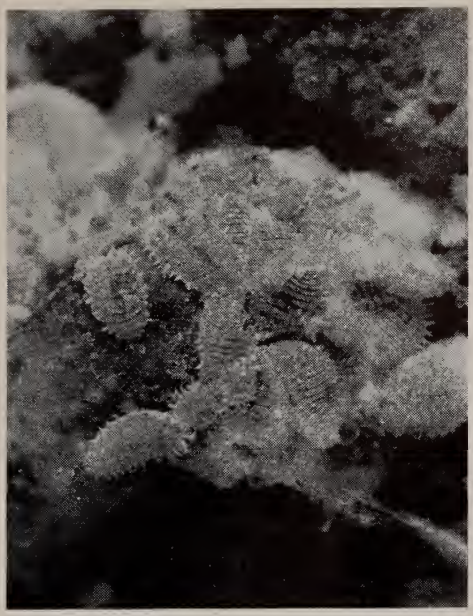
T. W. FISHER

INTRODUCTION

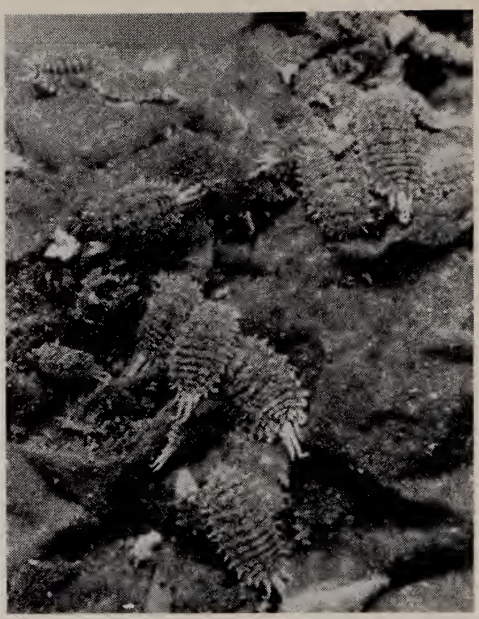
THE IMPORTANCE of mealybugs as pests of citrus in southern California may be surmised from the fact that between 1916 and 1930 sixteen insectaries for the production of *Cryptolaemus montrouzieri* Mulsant were established in citrus-producing areas. Insecticides effective against mealybugs were then not known, and during the period mentioned growers sustained economic losses from fruit drop and excessive smutting caused mainly by one or more of four species of mealybugs, depending on the locality. In decreasing order of importance these mealybugs were: (1) the citrophilus mealybug, *Pseudococcus gahani* Green, (2) the long-tailed mealybug, *P. adonidum* (Linn.), (3) the common (or citrus) mealybug, *P. citri* (Risso), and (4) Baker's mealybug, *P. obscurus* Essig.

Since 1928 all but the citrus mealybug have been reduced to, and held at, non-economic levels over most of their ranges by imported parasites and predators and by native predators such as brown lacewings (Sympherobiidae) and green lacewings (Chrysopidae). The citrus mealybug persists as a pest in coastal areas and, according to Mr. Willard Beckley, manager of the Associates Insectary in Santa Paula, Ventura County, it can best be kept under control by periodic colonization of insectary-cultured *Cryptolaemus* and *Leptomastix dactylopii* Howard (Beckley, 1960). Successful biological control of mealybugs on citrus resulting from establishment of several imported parasites and predators, as reported by Bartlett and Lloyd (1958), has been a major factor in the reduction in number of insectaries which produce "Crypts" and "Lepts," as these beneficial insects are colloquially referred to, so that now there remain in southern California only three insectaries owned by growers' cooperatives in Ventura County and an insectary at Chula Vista which is

[1] Submitted for publication Oct. 22, 1962.

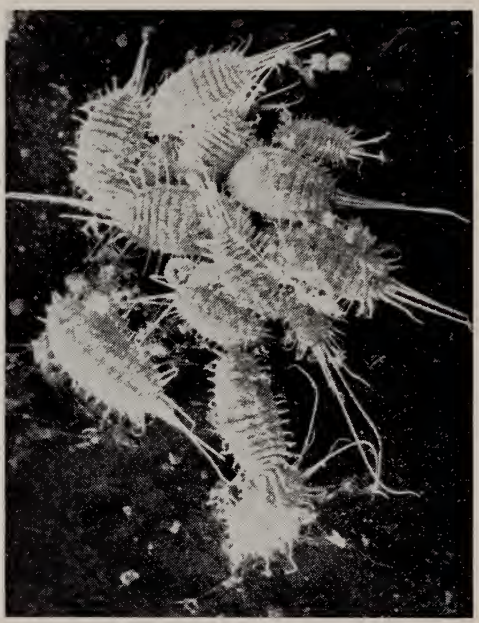


A. The citrus mealybug
Pseudococcus citri



B. The citrophilus mealybug
Pseudococcus gahani

C. The Baker mealybug
Pseudococcus obscurus



D. The long-tailed mealybug
Pseudococcus adonidum

Fig. 1. Four species of mealybugs occurring on citrus in southern California. Mature females attain a length of 3–5 mm in the laboratory.

operated by the county of San Diego.

The largest of these insectaries is the Associates Insectary, which was organized in 1928. Although a large inventory of chemical application equipment is maintained by this organization for the control of those citrus pests for which biological control in the area is inadequate, the primary reason for the existence of the insectary is the promotion of biological control (Beckley, 1956). It annually produces approximately 30 million *Cryptolaemus* and 40 million *Leptomastix* for biological control of mealybugs (chiefly *P. citri*) in its own 10,000 acres of citrus, and in the nearly 8,000 acres of another growers' cooperative pest control association.

The method of mass producing *Cryptolaemus* was previously reported by Smith and Armitage (1920 and 1931, out of print). Table 1 indicates some of the changes in production methods which have occurred in the last 33 years.

Table 1. **Changes in the *Cryptolaemus* program between 1930 and 1963**

Item	1930	1963
Host information:		
Species of mealybug	*Pseudococcus gahani; P. citri;* and *P. maritimus**	*P. citri*
Age when ready for *Cryptolaemus*	14 days	8 days†
Transfer medium	wild sunflowers, cocklebur, Australian pepper, wild tobacco, mallow	*Pittosporum undulatum, Schinus molle*
Variety of potato:	Idaho Russet	Bliss Triumph
Size of sprouts when infested	short	12 to 18 inches
Number of trays infested from one production tray	100–125; ready for use (self-infested) 35–40 days	25; ready in 8 days
Age of 12-inch sprouts	60 day average; 30 days in summer	21 days in summer; 30 days in winter
Watering schedule	10 days	5 days
Cryptolaemus production:		
Number of sting adults per tray	25	30–40
Sting (oviposition) period	18 days (collect 50% and re-use for oviposition)	12 days (70% recovery used once only; included in field releases)
Larval feeding period	12–20 days	12–27 days
Pupation period	8–12 days	7–14 days
Placement of burlap	tacked on wall and face of trays	tacked to racks in back and front
Life cycle of *Cryptolaemus*	45 days	30 days†
Number produced per tray	400 average—1,000 maximum	2,300 average
Progeny per female	30	115
Collection	10 per gelatin capsule	1,000 per tube
Longevity post-collection	5–7 days	held for 3 days maximum
Rate of collection per man-hour	2,000 per hour	60,000 to 100,000 per hour
Peak production	40 days after removing sting stock	30 days
Field release:		
Criterion for release	pre-release survey; use of trap bands; only medium or heavy infestations received *Cryptolaemus*	banding discontinued; insurance release by year around periodic colonization program; repeat release in persistent infestation
Rate of liberation	4,000 per hour per man	8,000–16,000 (or more) per hour per man
Minimum number per tree	10	20

* The locally available species of mealybug determined the species used in mass production programs. In Ventura County, *P. citri* has continuously been the host of *Cryptolaemus*; Citrophilus mealybug was concurrently propagated from 1929 to 1937 for mass production of its parasites *Coccophagus gurneyi* and *Tetracnemus*. During that period the Associates Insectary produced 151,500,000 *C. gurneyi*. In Los Angeles and Orange Counties, *P. gahani* served as the host of *Cryptolaemus* until the mid-1930's when *P. gahani* was brought under biological control by introduced parasites, mainly *C. gurneyi*. At that time *P. citri* became the sole host for the continued *Cryptolaemus* program.

† The somewhat shorter periods in the various life stages in 1963 probably result from operating the insectary at higher average temperatures ,and from changes in host species utilized.

Fig. 2. **The Associates Insectary at Santa Paula. Ten rooms in this building plus twelve rooms in another building accommodate the *Cryptolaemus* program. Adjustable shutters control amount of light entering culture rooms.**

CULTURE METHOD

THE CULTURE of any entomophagous insect concerns three interrelated biological factors: the host plant, the host insect (usually the pest species), and the parasite or predator.

In order to facilitate an insectary program it may be desirable to utilize substitute (unnatural) host plants and/or host insects. In the program described here, bleached potato sprouts serve as the substitute host plant of the citrus mealybug.

Production of potato sprouts

The use of potato sprouts as an insectary host for mealybug culture was first reported by Branigan (1916) who allowed potatoes to sprout beneath greenhouse benches. This technique, which opened the door to practical mass production, was later modified by Smith and Armitage (1920) who found that white sprouts which developed in subdued light and at temperatures of 70° to 72°F were more acceptable to mealybugs than were green sprouts.

Potato variety. The variety of potato best suited to mass culture of the citrus mealybug is the red-skinned **Bliss Triumph** seed potato, which produces

This bulletin **describes the mass production of two natural enemies of citrus mealybug as practiced by existing insectaries, most of which are financed by sizable groups of commercial growers who have sustained their programs of biological control of mealybugs for over 30 years. Information developed by the Department of Biological Control of the University of California, Riverside, leading to the improvement of insectary facilities and procedures is also included, as are biological data of value to insectary planners and management.**

sturdy sprouts highly acceptable to the mealybug and its natural enemies. Because of its tendency to produce tubers of variable size, however, the variety is becoming less popular with growers, who are turning to varieties of more uniform size.

Another red-skinned potato, the **Bounty,** has been tested for use as a host of citrus mealybug, but its sprouts cannot be readily fed upon by newly hatched crawlers. Half-grown mealybugs transfer satisfactorily to sprouts of **Bounty,** and consequently this variety can be used for salvaging mealybugs and *Cryptolaemus* which have left collapsed **Bliss Triumph** sprouts.

The **White Rose** variety is the most widely grown commercial potato in southern California, but it has poor storage qualities and its sprouts will not support mealybug populations as well as **Bliss Triumph** does.

Source. Because of the relative scarcity of **Bliss Triumph** seed potatoes in California, they are procured from the Nebraska Certified Potato Growers of Alliance, Nebraska. All *Cryptolaemus* insectaries in southern California pool their orders, receiving the first shipment in November (the Associates Insectary at Santa Paula uses 1800 bags of 100 pounds each annually). In February approximately 100 bags go to a grower in Chino, Los Angeles County, to be planted in March, and this local crop is harvested in July for distribution among the insectaries.

Storage. For approximately 3 months after harvest, sprout growth is insufficient

Fig. 3. **Cold storage room at Associates Insectary. Sacks are on a wooden platform which permits air to circulate beneath the potatoes, thus greatly reducing loss from decay.**

for mealybug production. To properly preserve tubers during this time they are stored in a room cooled to 36°F ± 1°. Here, fans provide circulation of air which reduces temperature fluctuation throughout the cold room and also reduces decay fostered by high humidity in dead air spaces, particularly those near the floor. Figure 3 illustrates such a storage facility, but does not show the false floor under which air moves.

Although sacks of top-quality potatoes (such as **Bliss Triumph**) may be stacked five or six deep, most varieties become bruised and crushed in the sacks on the bottom if so stacked. At the insectaries of San Diego County and the University of California at Riverside, potatoes are put into wooden crates when received. These crates, which accommodate 100 pounds of potatoes, are designed to permit maximum circulation of air around them. The crated potatoes are then put in a shaded outdoor area for two weeks, if temperatures are above freezing; this "air cure" toughens skins and improves storage quality. In the cold room, crates can be stacked as high as space permits, as weight is on the crates rather than on the potatoes.

Fig. 4. **Open-top crates used for potato storage. Design permits adequate ventilation and maximum use of storage area. Inside dimensions of the crate are 12½″ × 14″ × 22½″.**

Planting the potatoes

Trays. To minimize chances of disease, trays are sterilized in scalding water prior to re-use. Planting trays are made of redwood and their outside dimensions are 18″ × 18″ × 4″ (a planted tray of this size weighs approximately 45 pounds).

Soil. Soil used at the Associates Insectary in Santa Paula is a sandy silt obtained from a nearby river bottom (outwash). After a production cycle has been completed, the soil is dumped outdoors where it remains for 3 years before re-use in the insectary; during this "fallow" period it is layered with steer manure. The practice at this insectary has been to avoid sterilizing soil prior to re-use, although sterilizing the trays *and* the soil at the same time might be an improvement.

Plaster sand is used at the San Diego County Department of Agriculture insectary. It is inexpensive and is discarded after one planting.

Selection of tubers. Approximately 3 months after harvest, or when sprouts begin to appear, the tubers are ready for planting. Sound, whole potatoes of medium to large size are used, and 25 to 36 tubers are placed about ½″ apart on a ½″ layer of soil in the tray. They are covered with slightly dampened soil which is then leveled with a straight edge to form a flat surface ¼″ below the top of the tray; this provides a shallow basin and is important to the watering procedure which follows.

Immediately after planting, trays are placed on racks in a thoroughly cleaned room. Burlap strips are placed behind trays at this time, and will later serve as pupating sites for *Cryptolaemus*.

Sprouting conditions. As soon as trays are placed in the production room they are watered. Four or five days later

Fig. 5. **Planting potatoes. The metal striking tool is held as shown and pulled across the tray with its notched ends resting on the edges of the tray; this leaves soil at uniform depth below top of tray. (Note "eye" development of potatoes on left.)**

each tray receives 1½ pints of Semesan solution (manufactured by E. I. DuPont de Nemours, Wilmington, Delaware) as a deterrent to fungus growth; this is taken from a stock solution of 2 ounces of Semesan in 5 gallons of water. Trays should be filled brimful of water every 4 or 5 days, and watering should be continued after the sprouts have been infested and the parasite or predator "sting stock"[1] added. Temperatures of 70° to 74°F appear to be optimum for facilitating sprout growth from **Bliss Triumph** potatoes, and adequate air movement is necessary to prevent stratification of temperature and humidity.

Watering, which is something of an art, should be regulated to keep sprouts growing and turgid and should be discontinued when ovipositing adult *C. montrouzieri* (sting stock) are collected.

Relative humidity (R.H.) is influenced mainly by the watering program. In a production room with developing sprouts, temperatures of 78° to 84°F and relative humidities of 60 to 84 per cent have given good results, provided there is proper air flow through the culture room, to prevent stratification or accumulation of excessive temperatures or humidities.

The time from planting until infesting with mealybugs is usually 21 days in summer and 30 days in winter and early spring.

Control of light intensity in order to minimize leaf growth and chlorophyll

[2] Sting stock (= "sting bugs") : vernacular insectary term for ovipositing insects used to perpetuate or increase a population.

Fig. 6. **Potatoes 1½" or less in diameter are kept in soil-less trays and allowed to sprout. Several potatoes may be placed at random among collapsed sprouts on production trays in order to salvage mealybugs which would otherwise be lost.**

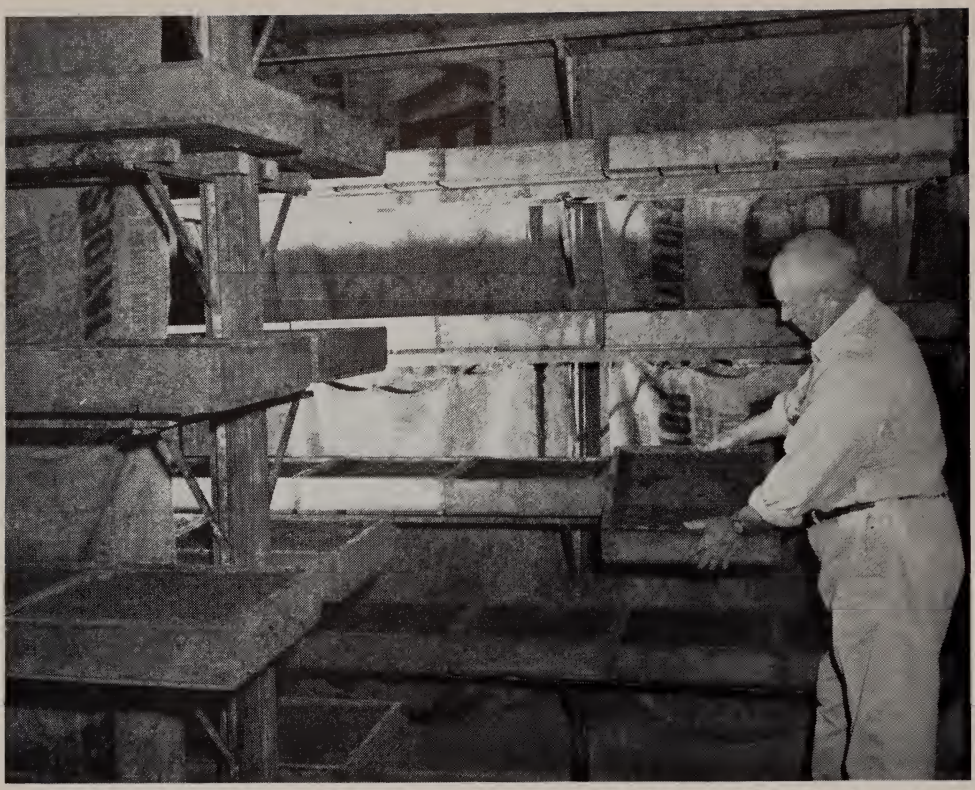


Fig. 7. **A newly started room. Note potato sacks suspended behind trays. Other burlap strips 6″ wide are fastened to the exposed sides of the trays later in the production cycle to afford additional pupation sites for *Cryptolaemus*.**

development is a critical factor in production of optimum (properly bleached) potato sprouts. Continuous weak light from windows causes sprouts to become excessively etiolated and too much light causes excessive leaf growth and development of chlorophyll. Light is admitted to the room for 1 week after planting, or until sprouts are 3 inches high, and then the room is darkened; if the sprouts continue to grow too fast light is again admitted for 1 or 2 days. Excessively long sprouts may be pruned to 12-inch lengths, but they should not be infested with mealybug crawlers until 5 or 6 days after pruning.

Effect of postharvest period on sprouting. Sprouts from potatoes which have been 9 or more months in cold storage are noticeably weaker than sprouts from 4 or 5-month-old potatoes,

Fig. 8. **Water must be carefully applied because of its effect on sprout development. Here, sprouts await infestation with mealybug crawlers.**

and older sprouts will collapse if infested with the same density of mealybug crawlers as used on younger tubers. Sprouts which develop after June 15 from potatoes dug the preceding fall are considered "old," and from this time until approximately January 15 the quality of sprouts declines and sprout collapse can become an increasing problem. Locally grown potatoes which are harvested in July do not sprout well until October. Between June 15 and mid-November (when using potatoes dug the preceding fall) it is standard practice to place uninfested trays of sprouts on the floor beneath the racks to catch and save the mealybugs. Placing small sprouted potatoes on the trays among collapsing sprouts will increase salvage as the salvage sprouts contain enough nutrient to sustain fairly sizable mealybug colonies long enough to permit *Cryptolaemus* to feed on them and thus to complete development.

Another technique for salvaging mealybugs which leave collapsing sprouts consists of placing small narrow trays of sprouting potatoes in front of and slightly below the larger production trays; the small trays are planted when production trays are initially infested with mealybugs, so that by the time sprouts of the latter begin collapsing those in the salvage trays have become large enough to support mealybugs. Early detection of sprout collapse is important, and measures should be taken immediately to salvage the threatened populations of the insects they sustain.

Mealybug production

Species utilized. Because the citrophilus mealybug had been brought under

Fig. 9. **Ideally grown sprouts in mealybug culture room. Crawlers produced from a typical room of 160 trays will infest 1200 to 1500 trays over a period of 2 weeks.**

biological control by 1929 (Compere and Smith, 1932), the mealybug which has been used in the mass production of *C. montrouzieri* and *L. dactylopii* for the past 34 years is the citrus mealybug, *Pseudococcus citri* (Risso). This mealybug is better adapted to the insectary program than are the other three previously mentioned species by reason of its shorter life cycle, higher fecundity, and suitability as a host for both *Cryptolaemus* and *Leptomastix*.

The initial culture. If a mass-production program is contemplated, the quickest and least expensive procedure is to obtain initial colonies of mealybugs from established insectaries; from 6 to 12 months will elapse before the insectary stock of mealybugs is large enough to support a practical mass-production program. The alternative is to build up a culture from field material, preferably utilizing species which occur locally. In either case it is of great importance to develop pure cultures of the host mealybug. Therefore, when fresh stocks of mealybugs are brought to the insectary the following points should be observed carefully:

- Do not introduce new stocks directly into a culture room.
- Consign new stocks to a room well removed from occupied insectary rooms.
- Collect crawlers[2] by placing the crawler-producing material in tight containers covered with organdie cloth and arrange containers so that the cloth faces a source of light. After crawlers pass through the cloth they can be blown or gently brushed onto glossy white cards. Most miscellaneous insects, parasites, and predators are too large to crawl through the organdie, but unwanted insects such as *Pauridia peregrina* Timberlake can easily be detected on the white cards and destroyed. The mealybug crawlers can then be passed to an attendant outside the quarantine area who takes the card to the infesting room. Crawlers are most safely removed from the card by snapping or striking it sharply while holding it inverted above a tray of sprouts. Because *P. peregrina* oviposits in very small mealybugs, it is possible to inadvertently transfer the contaminant to fresh sprouts. Accordingly, it is necessary to carefully observe trays infested in this manner. Ideally, each tray should be placed in an individual cage for a quarantine period of 30 days; if *P. peregrina* is seen, repeated screening of crawlers will be necessary.

Maintaining the culture. At the Associates Insectary, the citrus mealybug has been cultured with great success for over 30 years. Insectary temperatures there normally fluctuate between 65° and 85°F, and experience indicates that the optimum constant temperature for continuous culture of mealybugs lies between 68° and 75°F.

Normally, a room of ovipositing mealybugs (a "food room") produces crawlers for about 2 weeks; thus, it is customary to start a new room every other week. If room temperature has been in the low 70's, it will be necessary to raise it to 80°F for a few days in order to force production of crawlers. At temperatures near 70°F it is likely that the humidity will rise and sugar mites, *Rhizoglyphus* spp. and *Tyroglyphus* spp., will become troublesome; temperatures ranging from 76° to 80°F tend to prevent severe outbreaks of mites. In the *Cryptolaemus* production program at the Associates Insectary two mealybug food rooms provide crawlers for 22 rooms. Stock from one mealybug tray will infest from 20 to 25 trays of sprouts.

Transferring crawlers and infesting the sprouts. Crawlers are removed from producing-trays by allowing them to crawl onto freshly cut short leafy terminals, or "switches," of *Pittosporum undulatum* placed among the sprouts. *P. undulatum* in southern California is rela-

[3] Here "crawler" means the highly motile first instar mealybug. Insectary workers call this stage the "hatch" and older mealybugs leaving from collapsed sprouts are called "crawlers."

Fig. 10. Gathering crawlers from trays in the mealybug room preparatory to transferring them to fresh sprouts. Switches of *Pittosporum undulatum* serve as transfer media.

Fig. 11. Transferring mealybug crawlers to fresh sprouts. Sprouts are tied loosely together and *Pittosporum* leaves containing crawlers are placed in the nest thus formed. The sprouts shown are somewhat longer than is advisable.

tively free of insects which might cause problems in the insectary; *Schinus molle* (California Pepper) is also used in autumn. Approximately 6 hours after placing them on the trays of mealybugs, the switches are removed from the food room and placed on fresh sprouts in a production room where mealybug crawlers move onto the sprouts as the switches dry out. After 1 to 3 hours the switches are moved to fresh sprouts; switches can usually be moved three or four times before all crawlers have left them. Ideally, each sprout has several continuous lines of crawlers over its entire length, and the tip itself has crawlers well dispersed over the small leaves. When this occurs, switches should be moved to fresh sprouts. During the transfer period trays are not watered, light intensity is increased, and temperature is adjusted to 80°F; this combination of factors stimulates the crawlers' activity and reduces transfer time.

In a mealybug culture room myriads of surplus crawlers fall to the floor, especially on weekends when regular insectary routine may be suspended. Many fresh trays can be infested by salvaging these crawlers with freshly cut switches placed on the floor.

The type of floor used has a profound effect on the efficiency of the crawler salvage program. Heat from heated coils in the floor causes transfer branches to dry out too fast and this forces the crawlers to greater activity and they die in a few hours. Consequently, heat from a forced-air source via ducts is favored over radiant heat.

After sprouts have been infested, room temperature is maintained at a minimum of 74°F. At lower temperatures, the mealybugs develop so slowly that synchronization of subsequent steps in the mass-production program is disrupted.

The problem of premature hatching. Occasionally encountered in mealybug production, this problem seems to be caused more by high temperature than by any other single factor. To avoid it, it is important to observe the developing culture at least twice weekly and, if feasible, to have a maximum-minimum thermometer in each room of the insectary so that the operator is alerted to high temperatures. If daytime temperatures in the culture rooms are consistently well above 80°F it is advisable to either admit compensating cool night air into the building, or to provide air conditioning which will hold the average temperature within the range of 74° to 78°F. If premature hatching occurs, crawlers can be prevented from leaving the trays by placing sprouted tubers on the trays among the sprouts. Another method is to remove every third tray in the row and replace it with a tray containing long sprouts which can be carefully bent over to interlace with the over-producing sprouts of the adjoining trays. Another commonly used method is to add the ovipositing *Cryptolaemus* earlier than usual, after 5 or 6 days rather than after 10 or 12.

Cryptolaemus production

Biology. In the insectary, the life cycle of *C. montrouzieri* (Coleoptera: Coccinellidae) is completed in approximately 30 days at temperatures fluctuating between 74° and 80°F. Table 2 shows the duration of life stages at constant temperatures of 70° and 80°F and 60 per cent R.H.; the table represents results of 9 and 15 completed developments.

For this study, *Cryptolaemus* eggs less than 24 hours old were individually isolated under small glass cells with organdie cloth tops. By means of a ring of melted beeswax applied with a medicine dropper the cells were attached to green lemon fruits infested with several stages of the citrus mealybug. When the mealybug became noticeably reduced, the *Cryptolaemus* larvae were transferred with a 2/0 artist's brush to fresh cells covering enough mealybugs to supply food for 2 or more days. Throughout the observation period the *Cryptolaemus* larvae were

Table 2. **Life cycle of *Cryptolaemus montrouzieri* Mulsant at two constant temperatures and 60 per cent relative humidity**

Stage of life cycle	Duration	
	70° F	80° F
Egg	8–9 days	5–6 days
Larval instars		
1st	4–8	3–4
2nd	4–6	2–3
3rd	4–6	3–4
4th	5–6	4–6
Prepupa	6–9	2–3
Pupa	8–11	5–7
Days from egg to adult	43–47	28–29
Preoviposition period	not determined	4–5

provided with an overabundance of food, but in spite of this some mortality occurred.

To obtain daily oviposition records, nine teneral females were individually isolated with males, and each pair was transferred to fresh prey. As males died they were removed, but were not replaced with viable males. Table 3 shows the oviposition resulting from this procedure.

Fig. 12. **Citrus mealybug, upper; *Cryptolaemus* larvae, lower. In the laboratory mature citrus mealybugs average 4 mm in length, and mature larvae of *Cryptolaemus* average 7 mm in length (exclusive of wax-covered setae). These anesthetized individuals appear unlifelike in the photo.**

Table 3. **Oviposition and longevity records of nine female *Cryptolaemus* at 76–78° F**

Item	Performance			
	Total eggs laid	Average number eggs per day	Day on which 50 per cent of eggs had been laid	Longevity (days)
Range	194–729	7.11–11.04	11–31	27–70
Average per female	439.3	8.60	18	50.7

Sexual dimorphism. Anesthetization of adults permits observation of the differentiating sexual characters shown in figure 14. These characters were illustrated and described by Gomez Clemente (1932) but were incorrectly associated with the sexes. Another sex-determinative character is the color of the femora and tibiae of the prothoracic legs: those of the male are dominantly yellow and those of the female are dominantly dark grey to black.

Sexual maturity. A test at 76° to 82°F involving mating of 130 pairs of individuals of known ages for 2 to 3-hour mating periods showed that mated females could not produce viable eggs until the 4th day following emergence, and males had to be 5 days old before they could successfully inseminate the females.

Sexually immature males 1, 2, 3 and 4 days old made no attempt to copulate when placed with sexually mature virgins, although the virgins attempted to force themselves beneath the males. In a reciprocal test, 1, 2, and 3-day-old virgins were successfully mated by sexually mature males, but viable eggs were not deposited until the females were 4 or 5 days old.

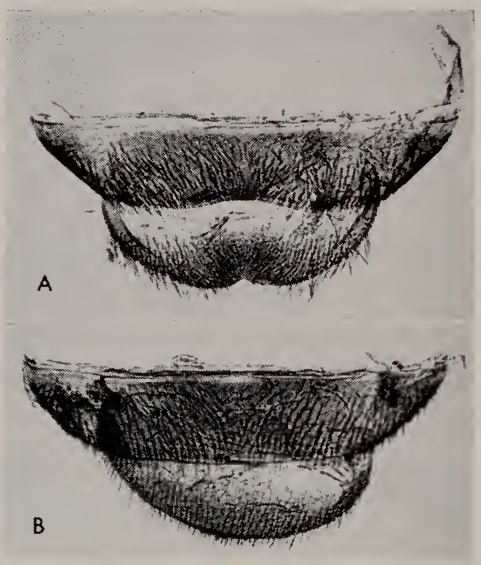


Fig. 14. **Photographs of ultimate and penultimate sternites of adult *Cryptolaemus*. A: Male. B: Female (ultimate segment is not completely exposed in this photo).**

Fig. 13. **Larvae of *Cryptolaemus* feeding in a colony of young *Pseudococcus citri* on a potato sprout.**

Because the beetles disperse rapidly once they are colonized in the orchard, it is possible that mating would be inadequate following the release of newly emerged adults, since males less than 5 days old are sexually immature. This difference in sexual maturation would be of considerably less significance when setting up new *Cryptolaemus* production rooms in the insectary, as the movement of the adults is confined to the room.

The author thinks that the number of recolonizations of groves could be re-

duced if more attention were paid to the ages of the adults released. Ideally, *Cryptolaemus* should not be taken from the insectary until mating occurs. This can be compensated for in part by making certain that a number of older *Cryptolaemus* from sting stock collections is included in each tube of fresh adults.

Sex ratio. Although counts of adults from rooms in various stages of production indicated some deviation from a ratio of 1:1, for practical purposes the adult population can be considered as 50 per cent female and 50 per cent male.

In the insectary, sting bugs are taken from "old" rooms and confined in close association in the production room so that chances for mating are good. However, dispersal could conceivably separate the sexes when released in the orchard, thereby necessitating further releases for adequate colonization.

Sting stock and handling of immature stages. When mealybugs are 8 days old, a sting stock of 30 to 40 adult *Cryptolaemus* (15 to 20 females) is placed on each tray in the darkened production room at 74° to 76°F, and their eggs are deposited liberally on the sprouts and the wooden trays. Twelve days later the wooden shutter is raised from the cloth-covered window in order to attract the adults, which are positively phototaxic, and during the following 4 or 5 days approximately 70 per cent of the adult sting stock can be recovered at the window. This method of insect mass production was termed the "limited-contact production method" by Flanders (1954). The sting stock is not used again in the insectary, but is included in field colonizations. Laboratory tests indicate that *Cryptolaemus* is capable of ovipositing for 6 weeks, with peak occurring during the 3rd week. After the sting stock has been collected, burlap bands are attached to the fronts of the racks to accommodate pupating *Cryptolaemus* and the room is again darkened.

Watering of trays during the oviposi-

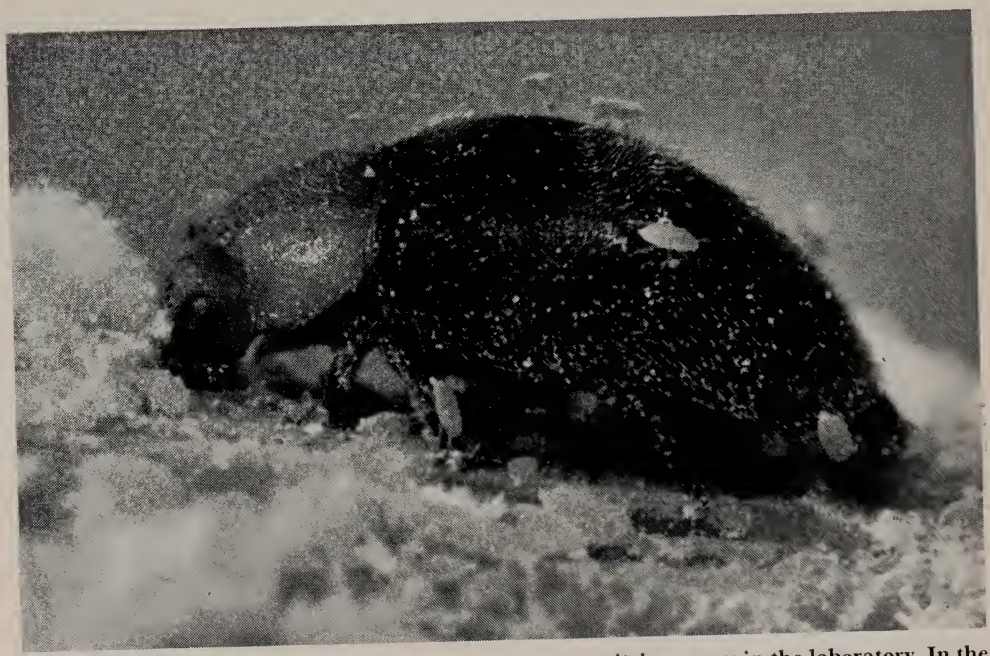


Fig. 15. **Adult *Cryptolaemus* feeding on a *Pseudococcus citri* egg mass in the laboratory. In the collecting tubes the beetles clean themselves of mealybug crawlers (visible on the feeding insect) so that the probability of transferring crawlers from insectary to field is virtually nil.**

Fig. 16. Cryptolaemus production room showing pupae attached to burlap strips, and on wall and ceiling.

tion period is continued until the sting stock has been collected; approximately 24 days will have elapsed since the sprouts were infested with mealybug crawlers (some of which will have developed to the egg-sac stage). During this 24-day period the *Cryptolaemus* eggs begin hatching, and many of the immature mealybugs are consumed by the developing beetle larvae which by this time are present in various stages of development. Active older larvae can force *Cryptolaemus* pupae off the substrate, with the result that many such dislodged pupae either die or emerge as deformed adults. Feeding larvae are strongly attracted to the mealybug colony, but fully developed larvae wander over the trays and racks looking for suitable pupation sites. Fresh burlap strips, as shown in figure 16, provide such sites and enable many larvae to pupate undisturbed by active larvae.

Because the citrus mealybug develops

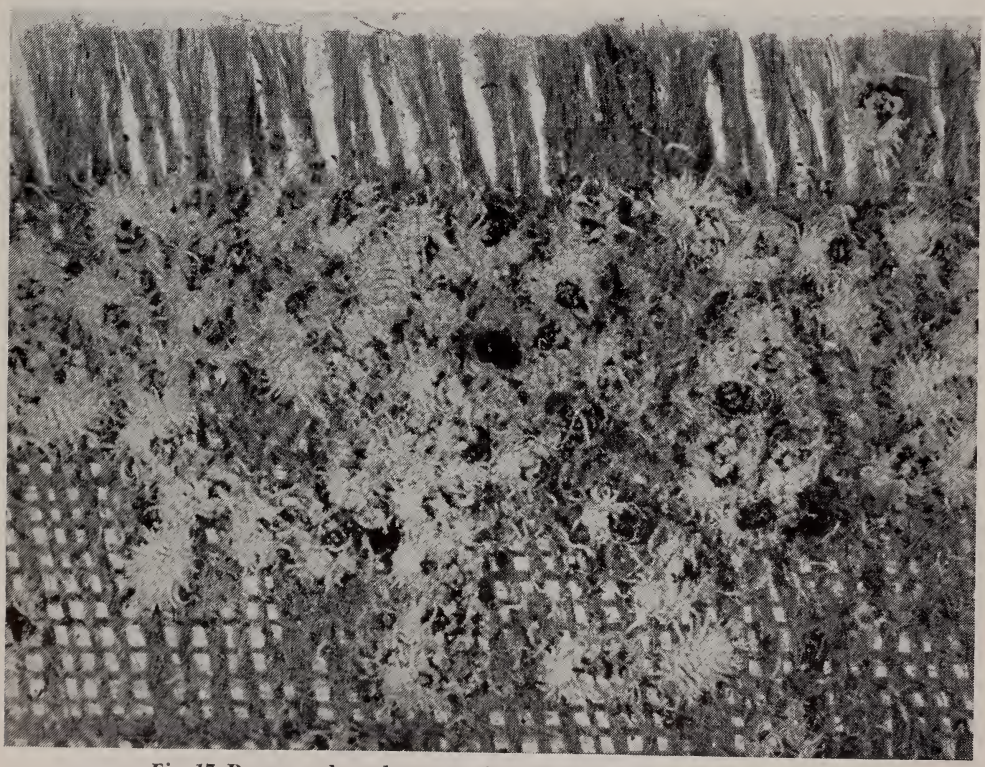


Fig. 17. Pupae and newly emerged *Cryptolaemus* adults on burlap.

rapidly, *Cryptolaemus* sting stock must usually be placed on the trays not less than 8 days after infestation—otherwise, the mealybug colony will develop faster than the *Cryptolaemus* can devour it and the sprouts will collapse from excessive feeding. Additionally, many by-passed mealybugs will have produced eggs and begun the second generation—and this generation would die from lack of food. If sprouts are inadvertently over-infested, their collapse can be prevented by placing the sting stock on the trays 5 or 6 days (rather than 8) after the infesting is completed.

Collection of adult *Cryptolaemus*. Eighteen days after removal of the sting stock the shutter is raised from the window. The emerging adult beetles fly toward the light and alight on a cloth which has been stretched tightly over the inside of the window opening in such a fashion as to prevent beetles from getting between it and the window. From two to six times daily (depending upon the rate of emergence) adults are collected into calibrated plastic vials by means of a broad flat scoop-funnel which is attached to the open top of the vial. The flat funnel is pressed lightly against the cloth and passed upward — when beetles are touched, they immediately fall into the vial. After 1,000 are collected, the funnel is replaced with a screened screw top.

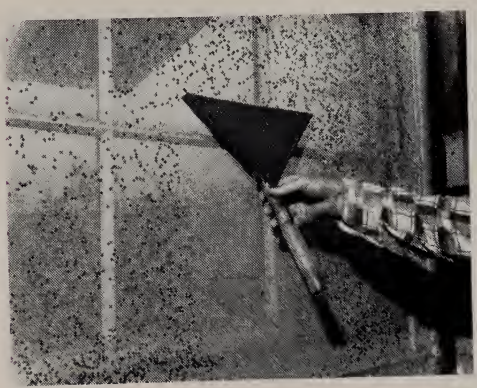


Fig. 18. **Collecting *Cryptolaemus*. Note the clean swath left by funnel as it was pushed upward against the cloth screen. There are approximately 1,000 beetles in the plastic tube.**

Fig. 19. **The clean, shiny appearance is typical of *Cryptolaemus* held in collection tubes, and is proof that mealybug crawlers are not transported incidentally to the orchard on the beetles.**

Production potential. Average production of adult *Cryptolaemus* per tray is 2,100 or more. Emerging beetles are collected for approximately 21 days; collection is discontinued when it drops to 2,000 per room daily. Adult *Cryptolaemus* may be safely held at 60° F in the collection vials for 12 to 18 hours prior to release; while they are in the vials they clean themselves of adhering mealybugs and thus carry none to the orchards. Mating also occurs in the vials.

Field colonization. In San Diego County, *Cryptolaemus* only is mass-produced (approximately 1,000,000 annually) and released in groves as the need occurs, at no direct cost to the grower. In Ventura County, insectaries are operated privately by growers' pest control cooperatives to achieve periodic colonization the year around as an insurance measure against mealybug build-up. The Associates Insectary reports that: "Our insectary production of mealybug parasites and predators has been held at a level [approximately 70 million annually] for the last several years. Experience has convinced us that regular liberations of beneficials for the control of mealybug will probably always be advisable, since

they do not seem to maintain themselves at a sufficient level to effect continuous control and for the lack of a satisfactory chemical spray program" (Beckley, 1960).

Normal release has changed from 10 beetles in a capsule per tree to approximately 20 per tree, metered by hand from the collecting tube originally containing about 1,000 adults. Only adults are released, and each tree receives from one to five releases annually, depending upon severity of the mealybug infestation. Usually, releases are made by men walking through the orchards twice daily at 8:30 to 9:30 a.m. and 3:00 to 4:00 p.m.

An orchard is considered ready for a *Cryptolaemus* release when the mealybug population moves onto the fruit (see figure 20), or if larvae from earlier releases are not in evidence in the colonies of mealybugs.

The heaviest releases are made in early spring and early fall for two principal reasons: (1) to clean up the mealybugs before insecticides drift into citrus groves from nearby vegetable plantings, thereby killing the mealybug's natural enemies, and (2) because *Cryptolaemus* will not oviposit during cool weather.

The presence of *Cryptolaemus* larvae among mealybug colonies 2 weeks after a colonization is an indication of a successful release, and control of the mealybug should be effected in two generations of *Cryptolaemus*. Absence of larvae necessitates a follow-up release of adults.

Fig. 20. **Infestation of citrus mealybug on Valencia orange in April. If similarly infested fruit are fairly common on several trees per acre in the spring, repeated releases of natural enemies will be necessary to control the pest by midsummer.**

Adults rather than larvae are released because larvae are more difficult to handle. Moreover, larvae are cannibalistic and heavy mortality occurs when rather large numbers are held in a narrowly confined place. When adults are released they disperse and seek out mealybug colonies for oviposition as well as for food.

Cost of production. Table 4 gives a comparison of production records for 6 years at the Associates Insectary. The annual gross cost to the cooperating growers in whose groves *Cryptolaemus*

Table 4. **Comparative production data for Associates Insectary, 1954 to 1960***

Year	*Cryptolaemus*				*Leptomastix*			
	Number produced	Average per tray	Number of trays used	Total cost (dollars)	Number produced	Average per tray	Number of trays used	Total cost (dollars)
1954–1955	28,854,000	2,610	11,055	31,465	33,798,000	17,778	1,900	26,628
1955–1956	23,131,000	2,128	10,865	30,146	36,957,000	17,598	2,100	27,954
1956–1957	34,000,000	2,600	13,095	35,309	40,000,000	17,333	2,300	25,553
1957–1958	32,464,000	2,500	12,985	33,626	40,464,000	19,270	2,100	15,766
1958–1959	31,007,000	2,378	13,035	36,027	56,722,400	24,662	2,300	16,888
1959–1960	30,540,000	2,132	14,320	36,234	47,509,000	22,620	2,100	17,166

* Data from Annual Reports 1955–1960.

and *Leptomastix* are colonized is approximately $11.50 per acre for three releases —$8.80 for the beetles and $2.70 for the wasps; this includes cost of producing the natural enemies and the inspection and supervision required to determine if additional releases are needed. Sale or trade of surplus natural enemies to growers outside the Associates Insectary cooperative brings the net cost to less than $10 per acre per grower-member.

Leptomastix production

Mass production of *Leptomastix dactylopii,* a valuable supplement to *C. montrouzieri,* has also been in progress at the Associates Insectary in Santa Paula since 1937, and periodic recovery attempts in that area verify continued presence of *Leptomastix.* However, its continued occurrence appears to be contingent upon the periodic colonization program.

At the Associates Insectary, *Leptomastix* is mass propagated in an insectary located approximately 200 feet from the *Cryptolaemus* insectary. The *Leptomastix* insectary is a complete unit and consists of ten rooms; of these, six serve simultaneously as potato sprouting and mealybug production rooms, one is a sting room, and one is a mass production room. For several years production has been mainly a one-man operation.

Although development of the mealybugs and parasites occurs in subdued light, ventilation of the culture rooms seems to be quite important, and fresh air is constantly forced through the insectary by means of a 1-horsepower fan.

In *Leptomastix* production, sting stock is not re-collected prior to emergence of its progeny; only the second generation of these parasites is released in citrus groves.

Biology. The most recent and complete account of the biology of the wasp *L. dactylopii* (Hymenoptera: Encyrtidae) is that of Zinna (1959) who quotes a communication from D. C. Lloyd of the Commonwealth Institute of Biological Con-

Fig. 21. Female *Leptomastix* ready to oviposit in a mature citrus mealybug.

Fig. 22. **Associates Insectary at Santa Paula, *Leptomastix* production building. Note adjustable opaque shutters over windows, and below them the openings (here closed) for screened collection boxes.**

trol: "... Oviposition is normally restricted to third nymph and adult stages of mealybugs. *Leptomastix* females will attempt oviposition in second instars but the normal end result of this is the impaling of the prospective host on the ovipositor. The finding of parasite eggs in these hosts is rare ... since the egg is deposited in the body cavity but attached to the derm by the long stalk, a small part of which protrudes from the host, the mechanics of the act are complicated and its execution difficult unless the host is of sufficient size and relatively stationary on the substrate." Table 5 contains Lloyd's data (Zinna, 1959) which indicate the host preference of *Leptomastix* and at the same time are indicative of the fecundity of the parasite when confined. Its *actual fecundity* during mass culture has not been determined, but it may be expected to fall within the maximum number of eggs a female can deposit per day (Flanders, 1961).

Although dissections by Zinna (1959) revealed that females have an ovipositional potential, or *inherent fecundity*, of 250 to 300 eggs, the progeny produced on citrus mealybugs, as expressed in small-scale, closely controlled laboratory tests, varied between 60 and 100. This marked reduction from the potential was ac-

Table 5. **Host preferences of *Leptomastix dactylopii* Howard on six species of mealybug***

Species of mealybugs					
Ps. citri	*Ps. maritimus*	*Ps. gahani*	*Ps. adonidum*	*Ph. gossypii*	*Ph. solani*
Number of progeny†					
363	2	0	4	17	27
344	0	5	7	11	18
379	0	1	3	5	38

* Data from Zinna, 1959.
† Progeny from 18 12-oz. jars in each of which 10 female *L. dactylopii* had been confined. In this test, fold increase on *P. citri* was 36.

Table 6. **Development of the life cycle of *Leptomastix* at 28° C (82.4° F) and 60–65 per cent relative humidity***

Stage of life cycle	Duration of stage
Egg incubation	32–48 hours
1st stage larva	20–30 hours
2nd stage larva	20–32 hours
3rd stage larva	25–35 hours
4th stage larva	20–30 hours
Prepupa	6–10 days
Pupa	7–8 days

* Data from Zinna, 1959.

counted for at least in part by three phenomena: superparasitism, phagocytosis, and synovigenesis. To elaborate: (1) More than one egg may be deposited per host (superparasitism). However, only one *Leptomastix* per host completes development, probably as a result of cannibalism among the parasite larvae within the individual host; (2) Eggs may be destroyed as a result of a physiological reaction of the host (phagocytosis); and (3) If a delay in oviposition occurs, the parasite absorbs those eggs which are ready to be laid (synovigenesis). It is presumed that partially or completely resorbed eggs either fail to hatch, or produce weakened larvae.

Zinna (1959) also reports that *Leptomastix* required 18 to 25 days for complete development at 28° C (82.4° F) and 60 to 65 per cent R.H. Table 6 shows the duration of the various stages under those conditions. It is reported by D. C. Lloyd that at 27° C (80.6° F) ± 2° C and 50 to 60 per cent R.H., emergence of males and females peaks on the 16th and 17th days, respectively, following egg deposition (Zinna, 1959).

Data from the Associates Insectary indicates that *Leptomastix* completes its life cycle in 28 days at 75° F ± 4°. The discrepancy between this figure and that reported by Zinna (1959) probably is the result of differences in temperature; the possibility exists, however, that strains with somewhat differing biological attributes are involved. This possibility becomes a virtual certainty when it is realized that the billion or more *Leptomastix* produced and colonized in California are all descendants of a single pair imported from Brazil in 1934 (Compere, 1939).

Handling potato sprouts. Production of potato sprouts and infesting them with mealybugs is the same as in the *Cryptolaemus* program. These functions are performed independently in their respective insectaries in order to minimize the possibility of inter-contamination of the beneficial insects.

The six rooms which serve a dual role as potato-sprouting and *Leptomastix* production rooms are worked in sequence so that one room is started anew each week. An additional two rooms are used as mealybug food rooms. Into a room containing sprouts that have developed to the

Fig. 23. Leptomastix oviposition cages. Exterior above; interior below.

Fig. 24. At the end of a *Leptomastix* production cycle sprouts have become dried and covered with mealybug mummies. Few mealybugs escape attack.

proper length (12 to 18 inches), mealybug crawlers are transferred on switches of *P. undulatum* taken from a room which was infested a month or more earlier.

Sting stock. *Leptomastix* sting stock is introduced as follows: Ten trays of sprouts containing 7 to 14-day-old citrus mealybugs are placed in a cloth-covered cage to which 32,000 wasps are added; this is a younger stage than the third instar reported as optimum by Lloyd on page 22 of the present publication. Twenty-four days later, or 4 days prior to the expected completion of the parasite's development, these 10 trays are moved into a darkened production room which contains 90 trays of 7-day-old mealybugs. Removal of trays from the sting cage at the proper time is important because the wandering habit of parasitized mealybugs can cause a considerable loss of potential sting stock. Parasitized mealybugs which leave the trays after they are brought into the production room are, of course, still part of the usable sting complement for that room.

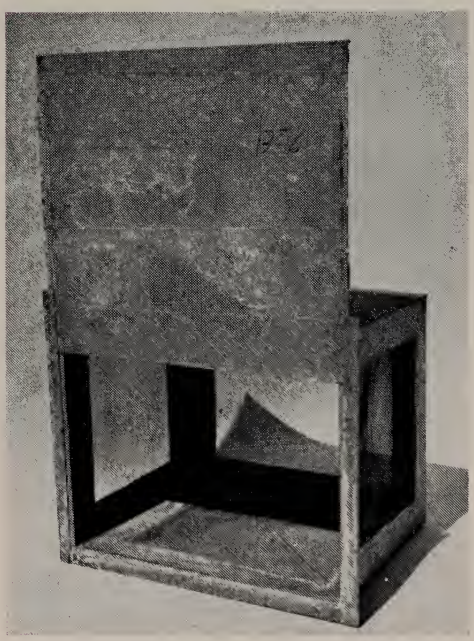


***Fig. 25. Leptomastix* collection box.**

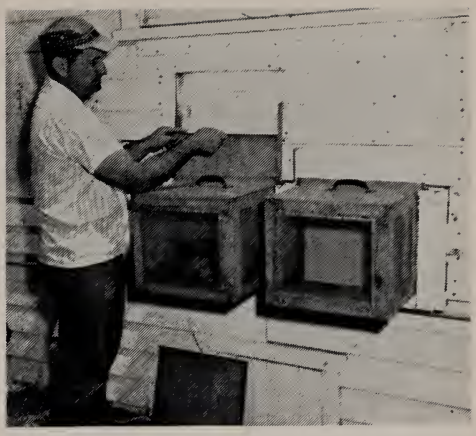


***Fig. 26. Leptomastix* collection boxes in use. Note double slides: the one nearest the building closes the opening in the wall—its exterior is painted white to match the building; the one being held goes with the box to the field.**

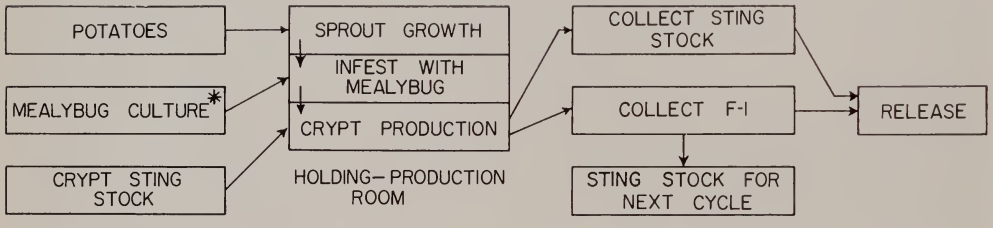
As parasites emerge from the sting trays, they begin ovipositing in hosts on the adjoining trays and gradually disperse throughout the production room. It is estimated that approximately 22,000 *Leptomastix* per tray are produced by this method; thus, each room has a potential of approximately 2,000,000 parasites. Figure 24 shows the extent to which host population is utilized by parasites.

Collection. Collecting the parasite involves use of its taxic responses, namely, positive geotaxis and positive phototaxis: adult *Leptomastix* tend to settle on the floor of a darkened room. In the outside wall of each room and at floor level are two or three openings 14″ wide and 12″ high. On the outside of the building, double metal guides along the sides of these openings accommodate metal slides which fit snugly and are used to close off the openings between collections. Flanges around the periphery of the collecting box fit into the outer guide. When both slides are raised, the light which then passes through the screened box attracts the parasites. Collection can be hastened if an air hose is used to blow the parasites along the floor toward the collecting boxes.

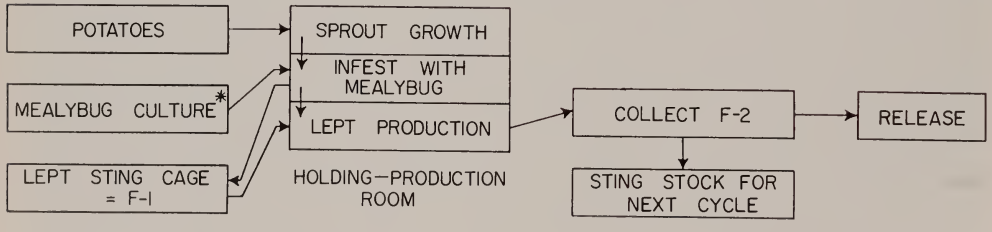
Fig. 27. **Photo taken from interior of *Leptomastix* production room, looking into collection box (left) attached to the outside of the building. Metal slide is in place over the opening at right.**

Fig. 28. **Parasites are allowed to escape from the collection box as an insectary worker carries it through the grove.**

When interior surfaces of the screened boxes are well covered with parasites, the opening in the wall is sealed off by pushing the metal slide in the inner guide into place, thus blocking egress of parasites from the room. A similar metal slide is



CRYPTOLAEMUS PRODUCTION



LEPTOMASTIX PRODUCTION

Fig. 29. **The relationship of various stages in *Cryptolaemus* and *Leptomastix* production cycles. Mealybug culture is perpetuated by periodically starting rooms for this purpose from the immediately preceding mealybug room. F-1 and F-2 are 1st and 2nd generations following addition of parent sting stock to mealybug host material.**

pushed down within the flanges on the open side of the box so that it can then be removed with the slide-cover in place.

Field colonization. Parasites from the Associates Insectary are transported in collecting boxes to member orchards up to 20 miles away, and are released by workers walking through groves while carrying the collecting boxes with the slides open about 3 inches. Experiments with vehicles carrying the boxes through the groves were discontinued because growers objected to the soil compaction resulting from repeated applications.

Production Costs. Between 1955 and 1960 the average annual production of *Leptomastix* was 42,575,000, and the average annual cost of production was $21,659. If this entire production were colonized on properties of co-op members, the average cost per acre per year would be $2.70. Table 4 provides more detailed information.

SANITATION

As mealybug, *Cryptolaemus*, and *Leptomastix* production cycles terminate, trays are removed from the rooms and the temperature is raised to 80° F or higher and held there for 7 to 10 days. (Insectary workers refer to this procedure as the "cook-out" period because the raised temperature hastens development and emergence of insects still concealed in the room). If contaminants were a problem during the preceding cycle, the room should now be treated with an appropriate insecticide, such as pyrethrum-in-oil spray which leaves no residue harmful to the subsequent insectary program. Fumigation with methyl bromide or HCN achieves a more thorough clean-up, but these or other fumigants should not be used unless room construction provides adequate safety. Following the temperature-raising, all surfaces and racks in the room are scrubbed with dry brushes to remove pupal cases and other detritus, and floors are scrubbed with soapy water and then rinsed.

Fig. 30. **Aftermath of a production cycle. Masks are worn while removing trays in order to prevent inhalation of dust composed largely of fine particles of mealybug wax, dead crawlers, and dried fine soil. A: Inside the production room. B: Loading truck in service corridor of building shown in figure 3.**

At the close of the production cycle, burlap strips used in the *Cryptolaemus* rooms are cleaned of exuviae by pulling them between two stiff brushes. The brushed strips are then rolled up, and are not re-used for approximately 3 months. During this period stickiness which may result from use of oily carriers of botanical insecticides will dissipate. If storage is a problem, the brushed burlap may be washed in warm water, rinsed, and dried for immediate re-use.

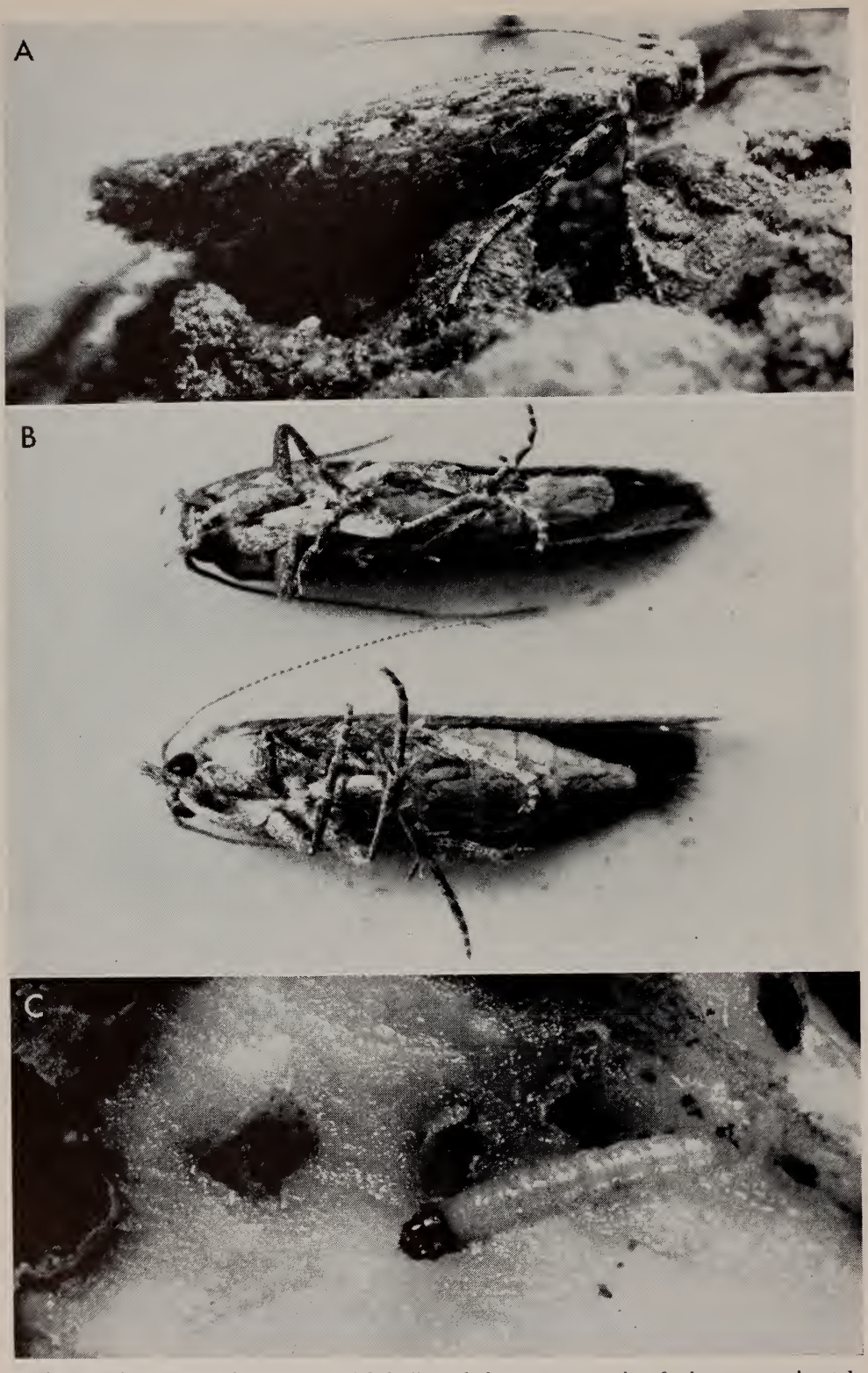


Fig. 31. The potato tuberworm. A: Adult. Length from snout to tip of wings approximately 8 mm. B: Ventral view of male (upper) and female (lower). C: Larva on cut-away section of damaged potato tuber.

CONTAMINATION

IN THE PARLANCE of the insectary worker, "contamination" usually refers to the intrusion of unwanted organisms into cultures of the host plant, host insect, or beneficial insect (other contaminants such as insecticides or cement dust will not be considered here). **Prevention is the best "cure,"** for once biological contaminants become established in the insectary it may be extremely difficult to control them, and the necessary drastic eradication procedures, such as fumigation, can seriously upset a production schedule. In fact, contamination could cause complete shut-down of one or more rooms, or of the entire insectary. Unfortunately for the insectary program, most new employees can be convinced of the soundness of the prevention principle only by having to deal with an actual contamination problem.

Contaminants of potato tubers or sprouts

While potatoes are in cold storage they are safe from insect attack, but once on racks to permit sprouting they are vulnerable to attack by the potato tuberworm, *Gnorimoschema operculella* (Zell.), and by mealybugs. Mealybugs on young sprouts at this time may be parasitized, and various predatory insects may be attracted before trays have been moved to a culture room. Accordingly, when potatoes must be handled they should be handled before workers enter culture rooms. One safeguard against tuberworm is to purchase potatoes from worm-free areas. Fungicides which minimize the build-up of root-rot organisms following planting may be of value also in preventing loss from that cause.

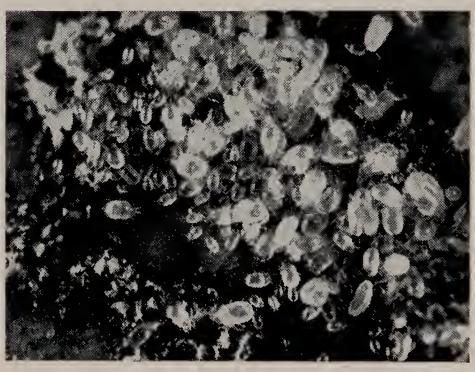


Fig. 32. **Sugar mites. Excessive humidity and temperatures near 70° F enhance multiplication of this insectary pest.**

Sprout collapse can lead to the proliferation of two secondary contaminants, sugar mites and fruit flies (*Drosophila* spp.). Trays of collapsed sprouts which are allowed to remain in the mealybug culture rooms are likely to permit great numbers of the sugar mite, *Rhizoglyphus* spp., to develop, particularly at temperatures of 70° F or lower. These mites move onto healthy sprouts and disturb young mealybugs and newly formed *Cryptolaemus* pupae. Large numbers of adult *Drosophila* constitute a nuisance factor for the insectary worker, and the slime produced as a result of the larvae feeding on the sprouts runs onto the soil of the trays and inhibits penetration of water. In addition, the mealybugs and the developing natural enemies may become trapped in the slime. Other contaminants of sprouts include aphids and whiteflies. These contaminants are occasionally troublesome on properly bleached sprouts, but are more so on sprouts starting to turn green and leafy because of improper control of light.

All the contaminants mentioned may be controlled without seriously affecting the wax-covered *Cryptolaemus* larvae by treating rooms with a pyrethrum-in-oil spray once or twice during a production cycle.

Contamination by other species of mealybugs

If the insectary contains only one host species, the problem of unwanted mealy-

bugs becoming established in culture rooms is greatly diminished.

Competitors of *Cryptolaemus* and *Leptomastix*

Of equal or even greater importance than the problem of unwanted mealybugs are the unwanted biological antagonists which may occur in the mealybug cultures and compete directly with *Cryptolaemus* and *Leptomastix* for food and space. Probably the most important are species of brown lacewings belonging to the family Sympherobiidae.

Parasites of mealybugs which may invade insectaries in southern California are *Leptomastidea abnormis* (Girault) and the previously mentioned *P. peregrina*. *L. abnormis*, the Sicilian mealybug parasite, was introduced into California in 1914 from Sicily and has persisted in the field as a parasite of *P. citri;* as an insectary contaminant it can cause the complete failure of the program. *P. peregrina* was introduced into California in 1949 from South China (Clausen, 1956) and it primarily attacks first instar *P. citri*. Insectary attendants claim that *P. peregrina* "torments" the immature *Cryptolaemus*, and it is apparent that even with an abundant food supply they develop at a much slower rate in the presence of a heavy population of *P. peregrina*. Notes by D. C. Lloyd regarding the biology of *P. peregrina* were included in a paper by Zinna (1960) and

Fig. 33. **Brown lacewing adult. Larvae feed on several stages of mealybugs; adults feed mainly on crawlers.**

state in part: "... Oviposition starts within a few hours of emergence from the host, and is usually completed in the first two days of life. Experiments show that at least 85% of the eggs are deposited during the first day. The oviposition act is seen in its most precise form during attack on active crawlers. The female *Pauridia* locates the *P. citri* crawler from a distance of a few millimeters, turns toward it, and lightly taps the nymph dorsally with the tips of the antennae for perhaps half a second. She then turns rapidly through an angle of 120° to 180° so as to face away from the host, and quickly inserts the ovipositor in the ventral surface of the mealybug.... There is no disturbance or dislodgment of these first instar hosts and a mealybug has never been seen impaled on *Pauridia* ovipositor as often occurs with other encyrtids attacking this host. When supplied with acceptable hosts the entire action of palpation, turning, ovipositor insertion, and egg deposition occupies approximately two seconds. The egg is deposited free in the body cavity.... Oviposition occurs in similar manner and with equal facility in settled first, second, and third nymphs of *P. citri*." According to Zinna (1960) larval development is completed in 11 to 12 days at 82.4° F, and the pupal stage lasts 16 to 18 days.

Judged by its low occurrence in field samples, *P. peregrina* in southern California is not considered an effective parasite. However, it decimates a portion of the mealybug population and as long as the production of *Cryptolaemus* and *Leptomastix* is maintained at the desired level, *P. peregrina* is considered by insectary workers to be a bonus feature of the insectary program. The author considers this a rationalization, for in fact each mealybug host of *P. peregrina* represents one less *Leptomastix* (a better parasite in that area of southern California) and the large numbers of *Pauridia* collected indicate that considerable quantities of prey were denied the *Cryptolaemus*

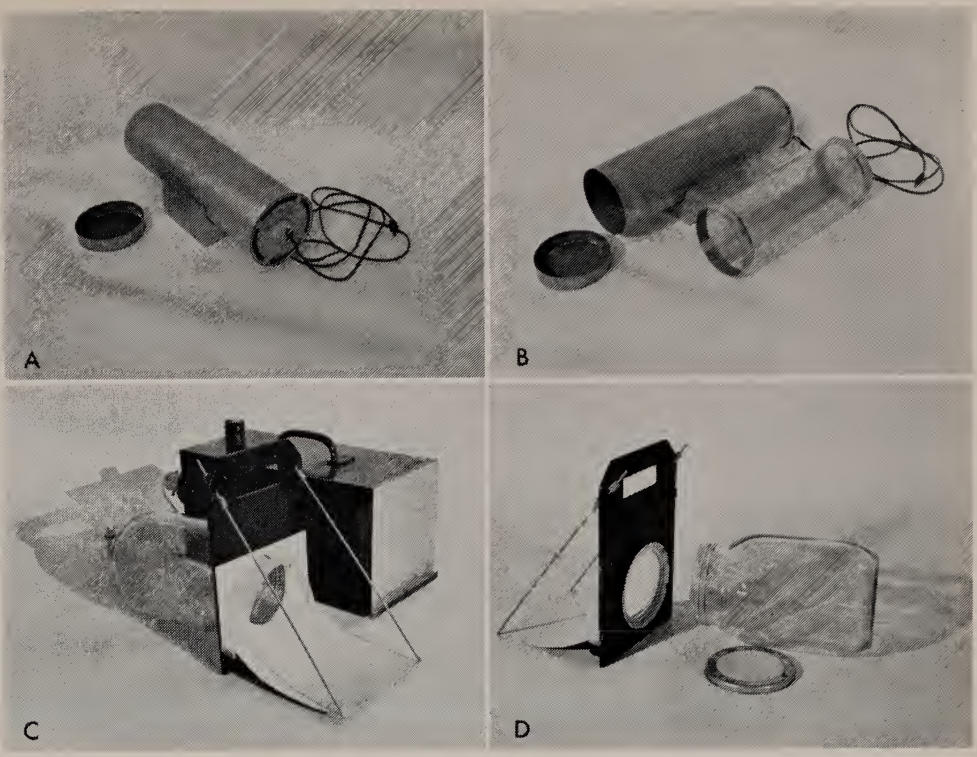


Fig. 34. **Light traps for collection of *Pauridia peregrina* Timberlake. A and B: Views of trap made from 6″ diameter galvanized stove pipe with a plastic insert to restrain the parasite. C and D: Adaptation utilizing 1-gallon jar insert to restrain the parasites. This trap was developed by Mr. James Cheney, Oxnard Pest Control Association. Virtually pure collections of *P. peregrina* result because the parasites must enter the traps by crawling through screen or cloth, the small mesh of which keeps out *Cryptolaemus* or *Leptomastix*. Additionally, entries of both traps are slightly elevated above floor to deny the mealybug crawlers access to traps. Twenty-five watt lamps provide the attractant and both traps are vented in order to discharge heat from the lamps.**

program. Nonetheless, special traps (see figure 34) have been designed to collect *P. peregrina* for release in the field. Primary parasites of *Cryptolaemus* have never been a problem in the insectary, and are of no known consequence in the field in southern California.

Once parasite contaminants become established in the mealybug culture it is difficult to eradicate them, but they can be controlled by periodically fogging the room with pyrethrum, which is not deleterious to the mealybug stock.

Sources of contamination

Because contamination is an ever-present threat to the insectary program it is important that the various means by which contaminants commonly gain ingress to culture rooms be understood.

From the field. The major insect contaminant from the field is the potato tuberworm. In the insectary, larvae of this moth burrow in tubers and destroy them by direct feeding and by causing increased vulnerability to disease organisms; the moths themselves also become a nuisance factor. If potatoes are purchased from tuberworm areas it is necessary to fumigate each lot (methyl bromide is commonly used) before they are brought into the insectary.

When potatoes are removed from cold storage, moisture condenses on them. Un-

less tubers are warmed to approximately 75° F, to dry their skins thoroughly, severe burning from the fumigant and subsequent and rapid deterioration will occur. A recommended procedure for warming cold-storage potatoes is to place them in a warm room, in single layers on or in trays, 12 hours before fumigating; sacks or crates of tubers require a warming period of 24 to 48 hours. Details regarding construction of a methyl bromide fumigation chamber may be found in Fisher (1963).

In the insectary. Most of the major contaminants and the remedial actions required to control them have been discussed in appropriate previous sections of this bulletin.

Insectary procedure. Workers should not leave a culture room without performing a decontamination procedure, and no one should enter a general office area without proper decontamination. Culture wings or buildings should be designed as self-contained units, so that personnel will not be obliged to move alternately through the various host and production rooms during the daily work routine.

Decontamination can usually be achieved by blowing pressurized air over the clothing, and inspection before a full-length mirror or by a co-worker is desirable. An added precaution is to change laboratory coats when leaving or entering a culture wing or building. Particular care should be taken to preserve the mono-specific character of mealybug rooms.

If two or more beneficial species are simultaneously propagated in the same building, improper room arrangement or screening and movement of personnel, or careless handling can result either in reciprocal or one-way contamination. To re-emphasize: *extreme caution must be exercised if material from the field is brought into the insectary.*

Visitors. Because a large insectary is of interest to entomologists, farm advisors, growers, and the public in general, guided tours are sometimes desirable. However, no one should be allowed to enter a culture room if he has been in the field within the preceding 4 hours, and only authorized insectary personnel should have access to mealybug rooms.

Other sources of contamination. Plants used in landscaping near the building or along adjacent walkways, roads, etc., should not be species on which mealybugs are common pests. The species of mealybugs on such plantings probably do not constitute a high hazard to the insectary cultures, but in time predators or parasites will find them and then find their way into the insectary—on the clothing or equipment of passers-by, or by direct flight through openings in the buildings.

Outside lights should be located so that insects attracted to them will not be apt to settle on or near doorways; inside lights should also be so located.

THE INSECTARY BUILDING

Floor plan. Figure 35 shows a suggested floor plan for *Cryptolaemus* or *Leptomastix* insectary similar to the Associates Insectary at Santa Paula. Each insectary building should be able to function as an independent unit in order to minimize the contamination hazard, as the area plan in figure 37 indicates.

If both *Cryptolaemus* and *Leptomastix* are to be mass produced, they should be housed in separate buildings. Since the basement area and refrigeration facilities indicated are adequate to supply potatoes needed for four insectary buildings, the second, third, and fourth buildings need duplicate only the above-ground portion of the plan shown.

Because labor is a major budgetary

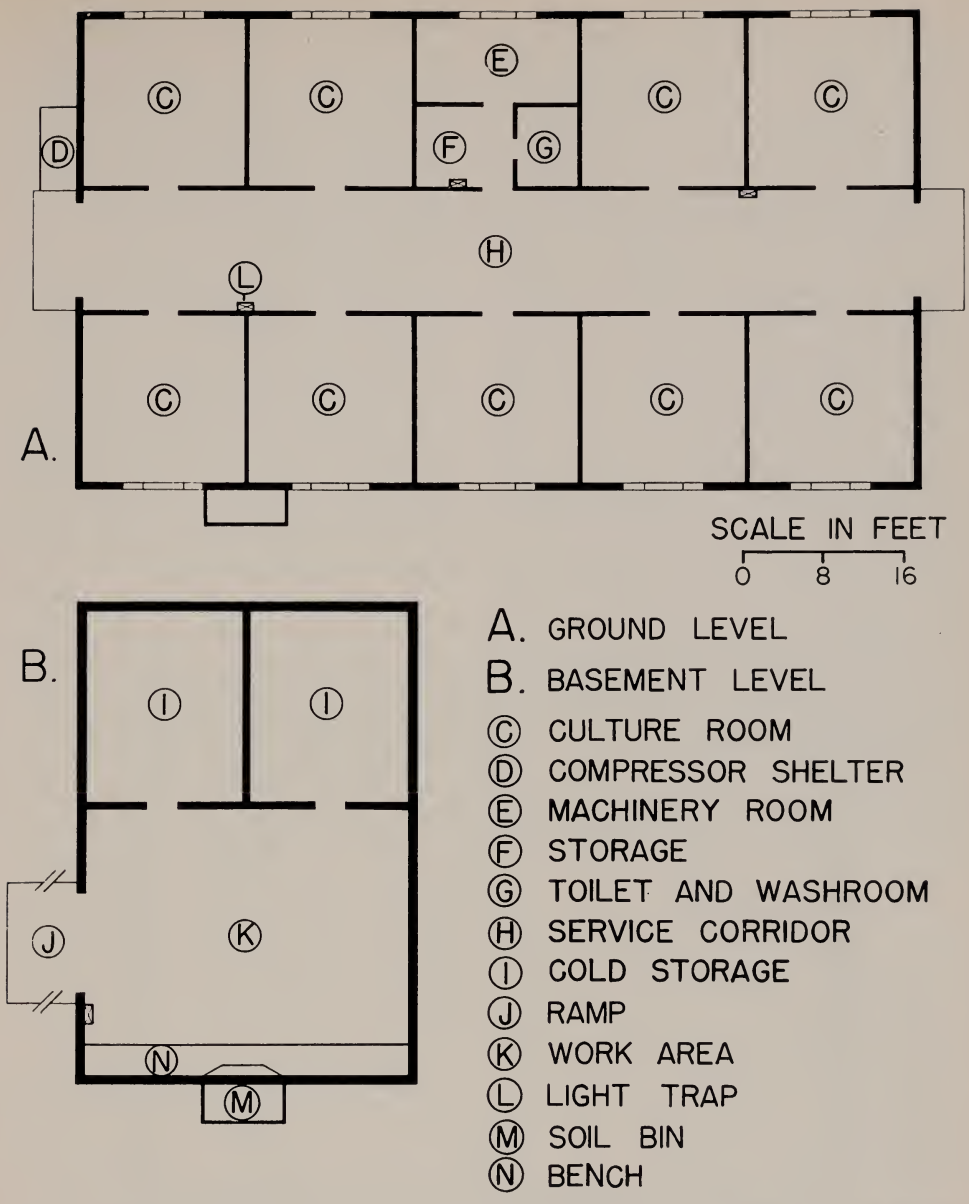


Fig. 35. **Suggested floor plan of an insectary for the production of *Cryptolaemus* or *Leptomastix*.**

item, labor-saving devices such as automatic lifts, rolling racks, powered carts, etc., should be considered during planning.

Depending upon costs of construction, available machinery, cost of maintenance and labor, etc., the floor of the ground level may be built to truck-bed height, in which case wooden floors probably would be utilized, or floors may be of poured concrete and therefore elevated only a few inches above grade level. From the viewpoint of maintenance and labor the latter seems preferable in a long-range insectary program.

An insectary building which permits

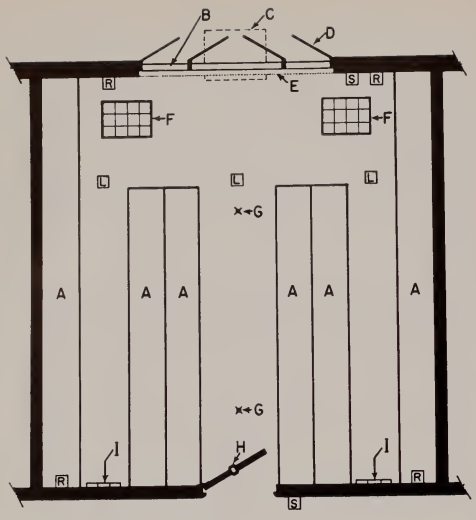


Fig. 36. **Room detail. A: Rack in 4 tiers. B: Window in three sections. C: Suggested location of optional room air conditioner above the window. D: Opaque shutters in three or four individually adjustable sections. E: Cloth screen. F: Air conditioning inlet in ceiling. G: Air pressure valve (overhead). H: One and one-half inch port in door for admitting fumigant. I: Air conditioning outlet in wall. L: Ceiling light. R: One-hundred and fifteen-volt duplex receptacle with weatherproof cover. S: Three-way switches to control ceiling lights. Switch outside of room with pilot light.**

direct access from the basement to the culture rooms above presents too great a contamination hazard. If such a building is contemplated, it should contain specially designed elevators and other features, such as insect-tight doors and decontamination rooms, necessary to minimize the possibility of contamination.

Most of the commercial *Cryptolaemus* insectaries in California use a truck to transport planted flats of potatoes from the planting area directly to the rearing rooms, and the same vehicle is used to move trays from exhausted production rooms. The truck, or powered cart, should have a bed large enough to carry a full room's complement (168 trays) according to the space available in the plan shown in figure 36. A newly-planted tray weighs about 45 pounds before watering, hence the full load weighs approximately 7,500 pounds—this weight (plus the weight of the truck) must be considered when the architect's working drawings are made. Two steel I-beams, one beneath each wheel track, with appropriate supporting pillars, will safely permit spanning the basement area.

It is possible that a windowless insectary (DeBach and White, 1960) would meet the requirements of a *Cryptolaemus* or *Leptomastix* program. Lighting for collection of the beneficial insects as well as for the control of sprout growth would be required, and it is quite possible that an "automatic" collecting unit consisting of a double light source could be employed to collect and count predators or parasites (Bedford, 1956).

Basement. This should be completely below ground in order to provide better temperature control for potato storage. Walls should be moisture-proofed and a floor drain or sump with pump may be required in most California areas. The amount of floor area will depend somewhat on its proposed use, but provision should be made for racks for the accelerated sprouting of potatoes and for air-conditioning equipment. In the plans shown, storage and planting of potatoes in an area well isolated from the contaminants (such as mealybugs) are prime considerations.

Cold storage facilities. The largest item housed in the basement is the cold storage facility, three walls of which may be formed by the walls of the basement. The other wall may be of masonry or well-insulated frame construction having appropriate footings and bonding devices provided during construction of the outer walls of the basement; these will permit the cold room to become an integral part of the basement structure. The top of the cold room may be formed by $2'' \times 8'' \times$ approximately 12′ joists spaced 12 inches apart. The interior ceiling may be $\frac{3}{8}''$ exterior grade plywood nailed onto the lower edges of the joists. Spaces between

joists should be filled with insulating material. Two 3′ × 6′8″ refrigerator doors may be installed in the long masonry wall, and the cold room may be divided in two by a partition of permanent masonry or insulated wooden frame construction, each chamber to be served by one door. The divided room permits flexibility of use and conservation of electrical power because one side only is needed during periods of reduced potato supply. A false floor and a recirculation system are needed for adequate circulation of air within the cold room, and cooling coils of relatively flat design mounted near the ceiling in each side will permit personnel to move freely inside. Both units may be served by a common compressor and condenser, which can be located outside of the building to prevent discharge of warm air into the basement.

Soil bin. Another facility needed in the basement is a bench on which potato planting can be done; a soil bin can be conveniently located above the bench.

Ground floor: *Culture rooms.* The culture rooms should each be approximately 16′ × 18′ and each should have a single door opening onto a central access driveway running the length of the building. These doors should be tight fitting and weather-stripped to prevent the entrance of light, and a fixed window approximately 8′ × 3′ should be on the wall opposite each door. The central 4-foot section of the window should be covered

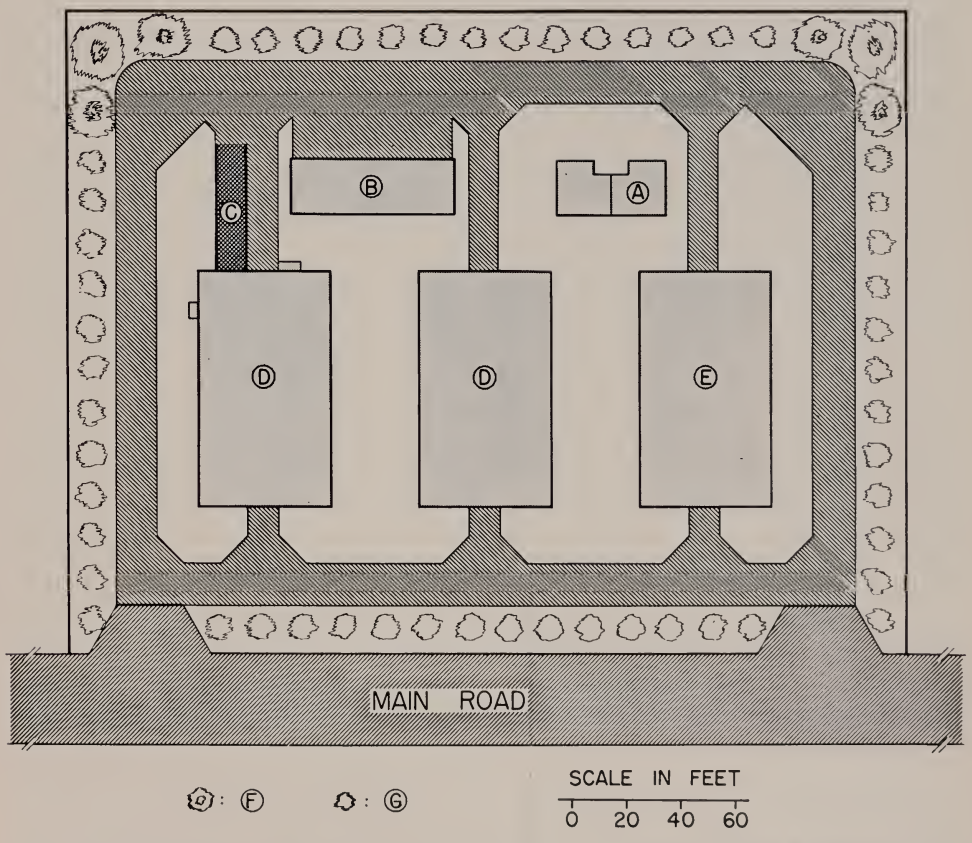


Fig. 37. **Suggested area plan of insectary buildings and related structures. Landscaping consists of plants suitable for transferring mealybug crawlers in the insectary program. A: Office B: Parking shed. C: Basement ramp. D: *Cryptolaemus* building. E: *Leptomastix* building. F: *Schinus molle*. G: *Pittosporum undulatum*.**

with a closely woven cloth stretched tight, its edges stapled and glued to the window frame so that it is 1″ to 2″ away from the glass. Such an arrangement will make a convenient collecting surface, particularly in the *Cryptolaemus* program. On the outside of the building an opaque shutter should be hinged so that it can tightly cover the window, thereby darkening the room. Flexibility in control of light is achieved if the shutter is constructed in three independent sections—two side sections each 2 feet wide and a central section 4 feet wide. The shutters should be independently controllable by means of cranks located within the room.

Each production room should have air lines with pressures of 60 to 80 pounds per square inch which can be used (1) to blow the insects from their resting places on the floor or walls toward the window or collecting boxes, or (2) as a source of negative air pressure when run through a Venturi tube in the event aspirator collection becomes desirable, or (3) to operate pneumatic temperature and humidity controls.

Because watering of trays is time-consuming, a system whereby several trays can receive water simultaneously is advisable. For example, instead of using a single outlet wand, the wand could be a longer pipe with four outlets spaced so that each centers on a tray. A permanently installed watering system would be ideal, but keeping the outlets free of wandering mealybugs and *Cryptolaemus* might become as time-consuming as watering trays individually.

Electrical facilities in all rooms should consist of at least two duplex receptacles with dust-tight covers, and ceiling fixtures so shielded that insects cannot contact them directly.

To help prevent contamination, permanently operating trap lights should be located near the ceilings in washrooms and general storage areas. Trap lights may consist simply of a bare 60-watt bulb in a glass box; insects enter through a slit and are killed when the hot bulb is contacted. In order to minimize traffic in the central office area, there should be an intercommunication system, as well as extension telephones to insectary buildings.

An outdoors area, which could be used for cleaning racks, trays, and other equipment, would be useful. Water and electricity outlets should be provided for this area.

Air conditioning. Specialized cooling equipment is not used in mealybug insectaries in coastal California. Here, outdoor temperatures from July through September normally fluctuate from 60° to 105° F; during this period, temperatures in the well-ventilated insectary range from approximately 70° to 95° F. However, rooms seldom exceed 90° F during the day if they are allowed to cool down at night by fans which pull cool night air through the insectary via properly screened openings.

Under normally fluctuating conditions as presently experienced, the life cycles of mealybugs, *Cryptolaemus*, and *Leptomastix* change as the seasons change. Thus the rate of development of these organisms cannot be predicted accurately, and the insectary worker must learn to cope with this variable.

A "time and temperature" method of mass production without precise air conditioning equipment is impossible, though desirable. If such equipment were utilized, the average fairly constant temperature selected for this program should be 75° F ± 1° F at a relative humidity of 68% ± 2%. Laboratory tests at Riverside showed that a constant 78° F caused considerable mortality to citrus mealybugs. Herein lies the hazard of constant temperatures near the upper limit of survival, for if malfunction occurs with the air conditioning machinery, a 3 or 4 degrees rise in temperature, if sustained for even a few hours, can cause great mortality to the mealybugs.

Ideally, the insectary man should have an interest in working with living creatures, a curiosity and willingness to try new approaches to production problems, and a considerable mechanical aptitude. He should also be amenable to discipline regarding personal habits, handling procedures, and recording of data—all highly important considerations in the prevention of contamination.

The good insectary worker is a dedicated artisan with a sense of right or wrong procedure regarding the handling of his charges—which, because they are living things, do not always behave in a perfectly predictable manner.

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